IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Garcia-Ladona

Serial No. 09/869,814

Filed: 05/07/01

Group/Art Unit: 1646

Examiner: JIANG, DONG

For : Binding partners for 5-HT5A receptors for the treatment of migraine

DECLARATION

1. I, Francisco Javier Garcia-Ladona, Ph.D., a citizen of Spain, hereby declare as follows:

   I am a fully trained biologist. Having studied biology at the Universidad Autonoma of Barcelona, and graduating in Biology, I received from 1987 to 1992 a doctorate (Ph.D.) from the University Louis Pasteur Strasbourg in Molecular and Cell Biology specialty Neurochemistry. I have been employed by Abbott GmbH & Co. KG and formerly by Knoll Aktiengesellschaft of 67061 Ludwigshafen, Germany for 11 years as research scientist in the field of CNS disorders including neuropsychiatric diseases and neurodegeneration. I have been working in the field of serotonin receptors since 1992. I am therefore fully conversant with the prior art.

   I am the inventor of the subject-matter disclosed and claimed in Appl. Ser. No. 09/869,814 and I am therefore familiar therewith.

2. I have read and fully understood the Office Action of May 22, 2003 and the references cited therein by the Examiner and conceived the experimental tests given in the specification of Appl. Ser. No. 09/869,814 or as described below.

3. Under my supervision, *in vitro* screening processes were performed in order to identify compounds which are suitable to treat cerebrovascular disorders such as migraine. Said processes comprised determining the binding affinity of candidate compounds for 5-HT5A and 5-HT1D receptors. In particular, those compounds whose binding affinity for 5-HT5A receptors is least 10-times higher than the binding affinity for 5-HT1D receptors were read out. *Inter alia* the following compound was identified having the binding affinities as indicated:

- HK02-01 ($K_i^{5\text{-HT5A}}$ = 3.3 nM; $K_i^{5\text{-HT1D}}$ >1000 nM)

Thus, compound HK02-01 binds to the 5-HT5A receptor with an affinity that is more than 10-times higher than its affinity for the 5-HT1D receptor.

4. Sumatriptan is currently used in the treatment of migraine. For sumatriptan the binding affinity for the same receptors are as indicated:

- Sumatriptan ($K_i^{5\text{-HT5A}}$ >1000 nM; $K_i^{5\text{-HT1D}}$ = 3 nM)

Thus, in contrast to the above compound HK02-01, sumatriptan binds to the 5-HT5A receptor with an affinity that is more than 10-times lower than its affinity for the 5-HT1D receptor.

5. Further, I evaluated whether the above compound HK02-01 is expected to be useful in a method for treating cerebrovascular disorders such as migraine. I asked Prof. Dr. Wolfgang Hanke at the Institute for Physiology of the University Hohenheim at 70593 Stuttgart, Germany, to investigate the effects of said compound in the retinal spreading depression.

6. Retinal spreading depression (rSD) is a well-recognized model for evaluating the efficacy of compounds in the treatment of migraine (see Fernandez de Lima V.M. et al. (1993) Brain Res. 614: 45-451; also refrerred to in the specification of Appl. Ser. No. 09/869,814).

Spreading Depression (SD) is an example of a physiological response of the neuronal tissue. It has been observed in all parts of the CNS, including the retina. Such SD waves after a proper stimulus (mechanical, chemical or electrical) propagate through the tissue with a velocity of 3–5 mm min^{-1} and consist of a short period of hyper excitation, followed by a longer period of complete suppression of electrical activity of the tissue involved. The waves are accompanied by a variety of other changes, like potential changes, changes in ion homeostasis, changes in ion channel parameters and in cellular volume. The SD is a transient phenomenon; the tissue completely recovers after several minutes.

A compound that significantly decreases spreading velocity, is expected to be effective in the treatment of migraine.

7. Prof. Hanke determined spreading velocity as follows:

7.1 Chemicals

7.1.1 Ringer

Retinas were perfused with standard ringer solution of following composition:

100 mM	NaCl
6 mM	KCl
1 nM	$MgSO_4$
1 mM	$CaCl_2\,2H_2O$
1 mM	NaH_2PO_4
30 mM	$NaHCO_3$
10 mM	TRIS
30 mM	Glucose

All substances except $CaCl_2$ were dissolved in aqua bidest. The pH was adjusted with HCl to 7,5, then $CaCl_2$ was added and pH was adjusted to 7,4. The used salts were obtained in p.a. from Fluka, Merck and Sigma in Germany.

7.1.2 Applied test compounds

HK02-01 and sumatriptan were dissolved in 1 % DMSO (Dimethylsulfoxid). 1 % DMSO alone had no effect on the investigated parameters of the rSD.

7.2 Set up

The set up was mounted on vibration-damped table. It consisted of an aluminum plate with four hollows for the petri dishes, a heating pad, a perfusion system containing two four-channel peristaltic pumps with tube system and one movable camera, that was mounted on a motor driven carriage. The retinas were perfused with ringer solution with a constant rate of 1 ml/min.

All experiments were stored on video tapes for later evaluation of the different wave parameters with adequate software. The video equipment contained camera video recorder, monitor and video processor.

7.3 Preparation of the eye-cup

For the experiments, chicken in the age from 5 to 21 days were used. After decapitation the eyes were removed out of the eye socket. Eyes were sectioned close to the equator and vitreous body was removed with tweezers. The posterior eye-cups were immersed in ringer solution. The eye-cups were glued each

in a petri dish and put into the set-up where they were perfused with ringer solution. Before the measurements started the retinas were allowed to recover for 30 min.

7.4 Protocol

For the velocity experiments several retinas were measured in parallel. The waves were elicited mechanically by gently touching the retina with a fine tungsten electrode. After each elicited wave the temperature was controlled. Then the camera was positioned over the next retina, where the next wave was elicited. After the same procedure has been carreid out with all retinas, the camera was moved to the first retina again. After 30 minutes recovery a new measuring cycle started. The first two waves were taken as controls with standard ringer solution. Immediately after the second measuring cycle the perfusion solution was changed. The standard ringer solution was then replaced by ringer solution plus the test compound. Five cycles with test compound were measured followed by two cycles with standard ringer solution to see whether the effects were reversible.

7.5 Data evaluation

The velocity was measured by measuring the time the wave front needed to travel over a defined distance on the monitor. The data were calculated in mm/min. The means of the controls were taken as y= 1; means of wave velocity under the action of test compound were compared to the control.

Significance was calculated with adequate software (Graph Pd Prism, t-test).

8. Prof. Dr. Hanke reported the following results:

8.1 HK02-01:

The spreading velocity of the waves is summarized in the following data table (relative spreading velocity (y) for all concentrations at different times of stimulation). The value at t = 0 is the mean of the controls and set to y = 1. Values are given as means ± SEM.

Time	10 µM			30 µM			45 µM			60 µM			75 µM			100 µM		
	y	SEM	n	y	SEM	n	y	SEM	n	x	SEM	n	y	SEM	n	y	SEM	n
00	1,00	0,0	8	1,00	0,00	4	1,00	0,00	4	1,00	0,00	4	1,00	0,00	1	1,00	0,00	4
30	1,06	0,04	8	1,10	0,04	4	0,99	0,06	4	0,98	0,03	4	0,99	0,00	1	0,98	0,14	4
60	0,99	0,04	8	1,08	0,04	4	0,91	0,03	4	0,88	0,04	4	0,80	0,00	1	0,80	0,11	4
90	0,98	0,04	8	1,04	0,03	4	0,86	0,05	4	0,82	0,04	4	0,75	0,00	1	0,27	0,16	4
120	0,98	0,04	9	1,00	0,04	4	0,86	0,05	4	0,78	0,03	4	0,71	0,00	1	0,14	0,14	4

HK02-01 significantly decreases spreading velocity. As can be seen, the higher the concentration of HK02-01 the more effectively the spreading velocity is decreased. HK02-01 is thus be expected to be useful for treating migraine.

8.2 Sumatriptan:

The spreading velocity of the waves is summarized in the following data table (relative spreading velocity (y) for all concentrations at different times of stimulation). The value at t = 0 is the mean of the controls and set to y = 1. Values are given as means ± SEM.

Time	400 µM			500 µM			800 µM			1 mM			1,5 mM		
	y	SEM	n	y	SEM	n	y	SEM	n	y	SEM	n	y	SEM	n
00	1,00	0,0	2	1,00	0,00	2	1,00	0,00	2	1,00	0,00	4	1,00	0,00	3
20	0,92	0,02	2	0,97	0,03	2	0,85	0,00	1	0,77	0,07	4	0,67	0,02	3
40	0,93	0,01	2	0,88	0,00	1	0,81	0,02	2	0,70	0,06	4	0,48	0,08	3
60	0,93	0,01	2	0,88	0,00	1	0,83	0,00	2	0,69	0,04	4	0,54	0,02	3
80	0,95	0,01	2	0,88	0,00	2	0,82	0,01	2	0,69	0,02	4	0,52	0,04	2
100	0,98	0,02	2	-	-		0,84	0,08	2	-	-	4	-	-	

Sumatriptan significantly decreases spreading velocity. As can be seen, the higher the concentration of sumatriptan the more effectively the spreading velocity is decreased. This confirms that sumatriptan is useful for treating migraine.

9. Further, from the above results it can also be seen that the compound having a higher binding affinity for the 5-HT5A receptor than for the 5-HT1D receptor (i.e. HK02-01) decreases spreading velocity more significantly than the compound having a lower binding affinity for the 5-HT5A receptor than for the 5-HT1D receptor (i.e. sumatriptan). This shows that migraine is associated with the 5-HT5A receptor. Moreover, since sumatriptan is known to be effective in the treatment of migraine, it is thus reasonable to expect that compounds having a higher binding affinity for the 5-HT5A receptor than for the 5-HT1D receptor (such as HK02-01) be even more effective in the treatment of migraine.

10. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishably by fine of imprisonment, or both, under section 1001 of title 18 of the U.S. code and that such willful false statements may jeopardize the validity of the above-identified application or patent issuing thereon.

Ludwigshafen, 17.11.2003

.....